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Free amino acids and biogenic amines in wines and musts from the Alentejo region. Evolution of amines during alcoholic fermentation and relationship with variety, sub-region and vintage

Paulo Herbert [a], Maria J. Cabrita [b], Nuno Ratola [a], Olga Laureano [c], Arminda Alves [a,*]

[a] *LEPAE—Departamento de Engenharia Química, Faculdade de Engenharia da Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal*
[b] *Laboratório de Enologia, Área Departamental de Ciências Agrárias, Universidade de Évora, Ap 94, 7002-554 Évora, Portugal*
[c] *Laboratório Ferreira Lapa (Sector de Enologia), Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Tapada da Ajuda, 1399 Lisboa, Portugal*

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Abstract

Characterization of musts and wines from different Alentejo sub-regions, varieties and vintages was made in terms of amino acid and amine contents, by HPLC with fluorescence detection of OPA/FMOC derivatives. The evolution of volatile and biogenic amines was also studied throughout 10 microvinifications.

No significant increase in the levels of total volatile amines was observed during alcoholic or spontaneous malolactic fermentation. While higher histamine levels were only found during the storage period, an increase in the concentration of tyramine was confirmed in red wines immediately after malolactic fermentation, which seems to be also the main origin of putrescine.

It was noticed that grape variety, region of production, and vintage can influence free amino acid and amine contents of musts and wines, although alcoholic and malolactic fermentations can override that effect.

Keywords: Amino acids; Amines; Alcoholic fermentation; Variety; Region; Vintage

1. Introduction

The presence of amines in musts and wines is well documented in the literature (Ough, Daudt, & Crowell, 1981; Mafra, Herbert, Santos, Barros, & Alves, 1999; Radler & Fäth, 1991; Zee, Simard, L'Heureux, & Tremblay, 1983), being biogenic amines of particular importance (Bauza et al., 1995). However, the processes that generate these amines, together with the factors that influence their quantitative and qualitative presence are in some cases not well defined yet and, sometimes, agreement is lacking between the published results.

Amines in wine may have two different sources: raw materials and fermentation processes. Some amines are already found in grapes, namely histamine and tyramine (Vidal-Carou, Ambatlle-Espunyes, Ulla-Ulla, & Mariné-Font, 1990), as well as several volatile amines and polyamines (Feuillat, 1998; Radler & Fäth, 1991).

Other studies showed that both alcoholic (Buteau, Duitschaever, & Ashton, 1984) and malolactic fermentations (Aerny, 1985; Kállay & Bódy-Szalkai, 1996; Vidal-Carou, Codony-Salcedo, & Mariné-Font, 1990) may produce amines in wines. Rivas-Gonzalo, Santos-Hernandez, and Mariné-Font (1983) and Vidal-Carou et al. (1990), stated that at least part (and sometimes all) of the final content of tyramine in the tested wines was due to alcoholic fermentation, although not confirming an increase in the histamine content during this process, nor finding any increase in histamine or tyramine levels during malolactic fermentation in 50% of the studied wines. However, Quevauviller and Mazière (1969) and Buteau et al. (1984) reported production of histamine during alcoholic fermentation, although Vidal-Carou et al. (1990) observed a highly significant correlation

* Corresponding author. Tel.: +351-22-5081883; fax: +351-22-5081449.
E-mail address: aalves@fe.up.pt (A. Alves).

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between levels of histamine and tyramine and lactic acid–malic acid ratio for red wines and a significant correlation in white and rosé wines.

Work concerning the factors that influence type and quantity of amines in wines, particularly biogenic, indicates lactic bacteria as the main cause for the significant generation of these substances, particularly some strains of *Lactobacillus* and *Pediococcus* (Moreno-Arribas, Torlois, Joyeux, Bertrand, & Lonvaud-Funel, 2000; Radler & Fäth, 1991); *Saccharomyces* species were shown to be weak producers of histamine by Lafon-Lafourcade and Joyeux (1975). However, some *Oenococcus oeni* strains possess decarboxylase activity capable of producing amines (Leitão, Teixeira, Crespo, & San Romão, 2000; Lonvaud-Funel & Joyeux, 1994), yeast extracts may contain noticeable amounts of histamine and tyramine (Blackwell, Mabbitt, & Marley, 1969), and some "non-*Saccharomyces*" yeasts (these may proliferate in the early stages of alcoholic fermentation) are capable of producing histamine at concentrations as high as 8.3 mg/l (Abad & Gómez, 1987). The presence of *Botritis cinerea* in grapes appears to influence both quantity and quality of amines in musts (Hajos, Sass-Kiss, Szerdahelyi, & Bardocz, 2000). Amongst others, vintage and technology employed (Sass-Kiss, Szerdahelyi, & Hajos, 2000), variety of grape (Soleas, Carey, & Goldberg, 1999), levels of amine-precursor amino acids in musts (Bauza, Blaise, Daumas, & Cabanis, 1995; Soufleros, Barrios, & Bertrand, 1998), and assimilable nitrogen content (Soufleros et al., 1998) seem to be relevant factors.

In this work, the levels of amines and assimilable amino acids in wines were studied regarding grape variety, region and vintage. To complement the work, an attempt was made to study the changes not only in the biogenic amine contents (histamine, tyramine, tryptamine, β-phenylethylamine, including the polyamines putrescine and cadaverine) but also in volatile amines during the alcoholic and malolactic fermentations.

2. Material and methods

2.1. Samples

A set of 209 samples from the Demarcated Region of Alentejo (including different sub-regions) was studied, comprising monovarietal wines from 1997 (20 samples) and 1998 (18 samples), monovarietal musts from 1998 (19 samples) and 1999 (20 samples), as well as the samples collected during the fermentations, that led to 1998 wines from the Évora sub-region (47 red and 85 white musts fermentations, collected at different stages of fermentation). To study the effect of variety, region and vintage, the following sampling scheme was employed: Monovarietal wines were produced by microvinification, with the main cultivars from the Alentejo region: Arinto, Perrum, Antão Vaz, Rabo de Ovelha and Roupeiro for white, and Aragonez, Moreto, Castelão, Tinta Caiada and Trincadeira for red. Trincadeira and Roupeiro came from the following Alentejo subregions: Évora, Portalegre, Borba, Reguengos, Redondo and Vidigueira, all the other cultivars being from Évora.

To study the evolution of biogenic amine contents during fermentation, only the samples from 1998 were used.

The volume obtained in each microvinification was about 40 l without temperature control, for red wine, and 20 l for white wine, fermented at 17 °C. Red grapes fermentation was conducted in steel microvinificators, without yeast inoculation and stems, in the presence of skin and seeds. White grapes fermentation was carried out in glass containers, with no yeast inoculation, stems, skins or seeds. Average length of white fermentation was 41 days (45 maximum and 34 minimum). At the beginning of alcoholic fermentation, 100 mg/l of SO_2 were added to both red and white musts, and another 50 mg/l after spontaneous malolactic fermentation completion in red wines, and after alcoholic fermentation completion in white wines. All red wines and Rabo de Ovelha white wine from 1998 showed spontaneous malolactic fermentation. The collected samples were immediately frozen and kept at −15 °C until analysis.

After the beginning of alcoholic fermentation, red and white wines were kept in glass containers (20 l) for ca. seven months at room temperature and ±17 °C, respectively. This can be considered a current procedure in the production of Alentejo wines, although white wines are usually bottled earlier (after three or four months). Following this period they were bottled and kept at room temperature until analysis. During the stage in glass containers, red and white wines were checked for free sulfur dioxide levels.

2.2. Analytical methods

Free amino acids (aspartate, glutamate, asparagine, glutamine, alanine, arginine, histidine, glycine, phenylalanine, tyrosine, tryptophan, γ-aminobutyric acid, serine, lysin, threonine, methionine, leucine, isoleucine, valine, proline and two intermediates of the urea cycle, ornithine and citrulline) and amines (ethanolamine, methylamine, ethylamine, histamine, tyramine, β-phenylethylamine, tryptamine, isoamilamine, cadaverine and putrescine) analyses were performed according to a previously developed HPLC method with fluorescence detection of the OPA/FMOC derivatives (Herbert, Santos, & Alves, 2001). Precision of this method ranged from 0.6% to 11.6% (relative standard deviation (%), RSD) for a standard solution (with an average amino acids concentration of 2.75 mg/l and an average amines